

The University of Chicago

FOUNDED BY JOHN D. ROCKEFELLER

THE ORIGIN OF HETERO- SPORY IN MARSILIA

A DISSERTATION

SUBMITTED TO THE FACULTY OF THE OGDEN GRADUATE SCHOOL OF
SCIENCE IN CANDIDACY FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

(DEPARTMENT OF BOTANY)

BY

CHARLES HOUSTON SHATTUCK

CHICAGO

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THE ORIGIN OF HETEROSPORY IN MARSILIA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 133

CHARLES H. SHATTUCK

(WITH PLATES III-VI AND ONE FIGURE)

Much morphological work has been done among the heterosporous pteridophytes, but they have not been subjected to experimental methods to obtain some suggestion as to the origin of heterospory. From certain phenomena that I observed in making a morphological investigation of *Marsilia quadrifolia*, it seemed possible that an experimental study might furnish some evidence as to the way in which the heterosporous habit began in this form.

Historical

The natural presumption has been that the Marsiliaceae have sprung from a homosporous ancestry, but there is little definite evidence to substantiate this view. CAMPBELL (4) has suggested that a near relationship exists between Marsiliaceae and Schizaeaceae. BOWER (2) calls attention to the striking morphological resemblance between the sporocarp of the Marsiliaceae and the spike of Ophioglossaceae.

JOHNSON (14), GOEBEL (11), and others have pointed out features suggesting that Marsiliaceae are not far removed from the homosporous ferns, but apparently no experimental work has been done which might show that the Marsiliaceae were originally homosporous and by what process they have attained their present heterosporous condition, or in fact how this condition has arisen in any other form.

In 1887 BUCHTIEN (1) called attention to the fact that in *Equisetum* the sex of the prothallium is controlled very largely by nutrition, and drew the conclusion that dioecious prothallia are an indication of incipient heterospory. This view will be discussed later.

The first literature bearing directly upon the origin of heterospory appeared in 1894. WILLIAMSON and SCOTT (24, 28), in their work on Calamaria, were the first investigators to offer a tenable theory. After an extensive study of two species of *Calamostachys*, in which

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they found abortion in all of the sporangia in *C. Binneyana* and in only one-fourth of the sporangia in *C. Casheana*, they suggested that the abortion of certain of the spores and the consequent increased nutrition of their surviving fellows may have been the physiological condition that ultimately rendered possible the development of megaspores.

In 1908 THODAY (27) added further and convincing evidence from *Sphenophyllum Dawsonii*, to that already furnished by WILLIAMSON and SCOTT (28). He shows that the size of the spore varies with the amount of abortion, and that while the average diameter of the spores in this form is $83\ \mu$, the maximum diameter where abortion is extensive is as much as $120\ \mu$, or nearly one-third larger. These are both striking examples of heterospory as it must have appeared in its incipency, and both show a very definite relation between the size of the spore and the extent of abortion.

Statement

Many have insisted that the only clue furnished as to the possible origin of heterospory is that found in Equisetum, where the prothallia are usually dioecious. This dioecism seems to be due rather to external than to internal conditions, as shown by HOFMEISTER (13) as early as 1855, when he pointed out that while the prothallia were for the most part dioecious, they could be made to produce either male or female sex organs, or both, by varying the external conditions. He states that archegonia may appear on late shoots of the so-called male prothallia; while SADEBECK (23) shows that antheridia may appear at a later period on the lobes of the female prothallia.

GOEBEL (12) says that "it is probable that in this, as in other cases, the male prothallia are those which have been insufficiently fed." BOWER (2) calls attention to the fact that the spores of Equisetum show no differentiation in size, or apparently in sex. CAMPBELL (3) also states (p. 453) that "external conditions influence the production of males or females as in the ferns, and that while the prothallia are normally dioecious, this is not exclusively the case." I have examined critically several laboratory cultures of the prothallia of Equisetum in which only antheridia were produced in the crowded portions of the sowing. In fact I have grown a number of cultures

in which I could not find a single plant bearing archegonia, though plants bearing antheridia were very numerous. This shows that *Equisetum* is only slightly more advanced in its tendency to produce dioecious prothallia than the ferns, and that this tendency is largely, if not wholly, controlled by external conditions after the spore begins to germinate, and probably not until the prothallium consists of many cells. This has also been mentioned by PRANTL (21) and others. Varied external conditions operating on the plant during the formation and maturation of the spore seem to make no difference in its size or tendency after germination to produce male or female prothallia. In other words, the plant makes no preparation in its spore for the production of female prothallia, the sex being determined long after germination and wholly by chance external conditions.

From a consideration of these facts it appears that the genus *Equisetum*, as we now know it, furnishes no clue to the origin of heterospory, because it makes no preparation which we can discover in the organization of its spores, either in size, shape, or extra storage of food material for the production of specially large gametophytes. In other words, the sex of the prothallium seems to be determined after germination and wholly by external conditions. In this particular we find it no farther advanced than the true ferns.

When we compare this form with one of the heterosporous pteridophytes, for example *Marsilia*, we find that the latter plant begins to prepare a few special spores as soon as the tetrad divisions have occurred, which are to produce the female prothallia. These few large spores are produced at the expense of many aborting ones whose substance is gradually absorbed by them. Moreover, these large spores are formed, under normal conditions, in the oldest and most favorably placed sporangia (*figs. 1, 2*), thus showing that here the sporophyte makes definite preparation for the production of spores which are to produce female prothallia.

In the microsporangium every one of the 16 mother cells (*fig. 3*) produces four functioning microspores, 64 in all; while in the megasporangium only one spore matures out of 64, all of which seem to be identical in every particular when the tetrads are first formed (*fig. 4*). A closer examination reveals the fact that this one megaspore does not gain the ascendancy without a struggle. In fact, many instances

were found in which there was sharp rivalry between two or more enlarging potential megaspores (*figs.* 5-9). Sometimes this occurs in the same tetrad, occasionally all four enlarging (*figs.* 5-7); sometimes two members of the same tetrad enlarge (*figs.* 5, 8, 9); and sometimes one each of two or more tetrads enlarges. Yet in the end, one centrally placed spore always gains the ascendancy, the others becoming abortive.

This apparent plasticity of *Marsilia* led me to take up a further study of it grown in the greenhouse, and under such conditions that a very careful record of results could be kept. My object was to see what effect varying conditions of light, heat, moisture, etc., might have on the production of the two kinds of spores.

Even among seed plants the development of the megaspore is by no means so uniform as is that of the microspore, even the formation of the well-known tetrad being dispensed with in many forms, such as *Lilium*, where the mother cell forms four nuclei but fails to develop walls. These nuclei are thought by many to represent four megaspores. We find also that from a single megaspore there may come a varying number of cells within the embryo sac, as found by CAMPBELL (5) and JOHNSON (15) in *Peperomia*, where 16 nuclei are formed; and by the writer in *Ulmus americana* (25), where 8 to 16 nuclei are formed; and while 8 is the usual number for angiosperms, some plants fail to form even so many, as was shown by Miss PACE in *Cypripedium* (19), where only four are formed.

Also, where the row of four megaspores is formed, there is often a sharp struggle for the mastery; sometimes the lower one, sometimes the upper one, and even one of the middle ones finally functioning as the embryo sac. This is well shown in such forms as *Diospyros*, in which Miss HAGUE, in an unpublished paper, finds the above conditions. Occasionally two megaspores function, forming two embryo sacs, as shown by ERNST (10), and by the writer in *Ulmus* (25) and *Pinus* (unpublished).

The nuclei within the sac also contend as to which will function as the egg, as shown by CHAMBERLAIN (6) in *Aster*, and by Miss OPPERMAN (18), and by the writer (25). In contrast with the above varying conditions of the megaspore we find a very constant method of development for the microspore, the mother cell nearly always

forming four spores (rarely 3 or 5), and a very large percentage of these developing, which are very constant in size, number, and behavior.

After reviewing these facts we must conclude that the megaspore condition is a derived condition, that is, derived from the original homosporous condition of the ferns; that it is therefore more recent, and consequently more plastic, and more likely to yield interesting variations when subjected to experimental methods.¹ With these general facts in mind the experimental work was begun.

Methods

In attacking this problem it was first necessary to determine definitely at what stage in its development the contents of the sporangium give positive evidence as to whether a megaspore or microspores are to be formed. JOHNSON (15) determined that the megasporangium is the first sporogenous tissue to be differentiated, but did not trace the development much farther. This has been done, and we find that all sporangia have identically the same development until the tetrads are formed (*fig. 4*), at which stage a very slight difference is observed.

In the older sporangia (*fig. 2*), which are also the most centrally located, the four young spores of each tetrad show a marked tendency to hang together by strong protoplasmic strands, as figured by STRASBURGER (26) and shown in *figs. 5-10*. These strands are the first recognizable morphological feature by which one can determine that a sporangium is to form a megaspore rather than microspores. They persist until the megaspore is quite mature, and in very many instances can be seen on the papilla of the germinating megaspore, which still subtends the three abortive members of the tetrad (*fig. 10, a*). This condition was figured by WILLIAMSON and SCOTT (28) for *C. Binneyana*, although they did not attempt an explanation. In the case of sporangia which were to form microspores it was observed that the protoplasmic strands are not so strong, and that the young spores, while held together during their early development, are in the end separated completely from each other. The fact that the megaspores are held together by stronger protoplasmic strands was first noticed by SACHS in 1866 in *Pilularia globulifera* (22).

¹ After this work was well under way Miss PFEIFFER (20) reported cases in *Azolla* where two megaspores have matured instead of one, which is the normal number.

The cause for the varying behavior of the strands in the two kinds of spores was not determined, but the establishment of this fact suggested that any experiments on the plant which might affect nutrition and growth ought to have a more marked influence in determining the kind of spore formed if applied when the sporocarps contained sporangia about ready to form tetrads. The various methods herein described were all found to give the best results if applied at this time.

Various cultures were grown in ponds, in the open air, and in the greenhouse. Careful records were kept covering such points as rate of vegetative growth, color, and vigor of plants, length of petioles, length of time before the appearance of sporocarps, the nature of the sporocarp, the causes producing the largest and most abundant sporocarps, and the causes of blasting of sporocarps and of their complete suppression (details of these cultures will be published in a later paper).

The experimental work was begun in Chicago, June 20, 1905, and continued until the latter part of September; it was repeated again during the same period in 1906; and then carried on continuously from June 1907 until the present time.

On June 20, 1905, I found *Marsilia quadriifolia* growing in Hull Court pond at all depths from 30^{cm} beneath the surface to 20-25^{cm} above it on the banks. It was in a most luxuriant condition, some of the rhizomes being more than 2^m in length. There was also one tank of growing plants in the greenhouse. Sods from the pond were taken up and transferred to the greenhouse, where they were placed in four large tanks (60 by 90^{cm} and 30^{cm} deep). These were supplied with soil (a rich black loam) spread evenly over the bottom to a depth of 10^{cm}, in which the sods were transplanted at one end of the tank and covered with water to the depth of 5^{cm}. In a few days these tanks were elevated 10^{cm} at one end. This left the soil at the upper end out of water, but submerged the plants, which were at the other end, about 10^{cm}. The tanks were then numbered 2, 3, 4, and 5, and placed under as great a variation of light as was possible to obtain in the greenhouse. All other conditions were kept as nearly uniform in all the tanks as possible, but on clear days there was considerable variation in temperature, as shown by the table of temperature readings.

TABLE I

LIGHT READINGS TAKEN IN OPEN AIR AND IN WATER AT DEPTHS OF 2.5, 5, AND 10^{cm}, AND ALSO IN NORTH AND SOUTH ENDS OF GREENHOUSE

These readings were taken with a solio-paper actinometer and show approximately the varying intensities of the light where different cultures were grown.

1907	Time	Weather	Open air	UNDER WATER			GREENHOUSE	
				2.5 ^{cm}	5 ^{cm}	10 ^{cm}	South end	North end
September 14	3:45	clear	15 sec.	..	..	..	35	65
September 20	12:20-12:40	clear	10 sec.	14	20	..	..	...
September 25	2:00- 2:20	clear (?)	20 sec.	..	..	35	70	210 (shade)
September 26	2:30- 2:40	cloudy	45 sec.	..	..	70	120	170

The plants in the best lighted positions recovered from the effect of transplanting more rapidly and grew much more vigorously than those in more subdued light. This was shown by the rate of growth of rhizomes, which under the most favorable conditions was as much as 12.5^{cm} daily; while for those in the most subdued light 6^{cm} was the maximum daily growth. The color of the plants in good light was also a darker green and the thickness of the leaf blades, rhizomes, and petioles was also greater. Neither the length of the petioles nor the size of the leaves was so great as those of the shaded plants.

On August 6, the first sporocarps were discovered in tank 5, which had been placed in strong light; these were evidently several days old and must have been evident as early as August 3. On August 8, tank 4 showed some sporocarps that had probably been in evidence about two days; while in tank 3 the very first indication of sporocarps was noticed. Tank 2 was in diffused light except for about two hours in the middle of the day, and while the plants grew well they stubbornly refused to fruit either above or below the surface of the water. Finally on August 17 some sporocarps were found in this tank out of water. These soon turned brown, however, and on sectioning were found to be blasted. This tank continued to produce a few blasting sporocarps for 18 days, but it produced good sporocarps as soon as it was placed in a strong light.

Influence of water

It has been observed that *Marsilia quadriifolia* fruits most profusely at the surface or a few centimeters above the surface of the water.

It fruits sparingly under water, and at a depth of 10^{cm} very few sporocarps appear. I observed that most of these blast when about half grown.

In my greenhouse cultures in 1905 I found that all the first sporocarps that appeared in tank 1 blasted when about half grown, except two that happened to be about 8^{cm} higher than the others, due to the uneven surface of the soil in the tank (*fig. 12, m*). After the rhizome passed over this elevation and again sank into 8-10^{cm} of water, the sporocarps began to blast as before. In order to be sure that this was not accidental, on July 5 I lowered the water in this tank until it was not more than 1^{cm} in depth; and then nearly all the sporocarps matured. On July 11 I raised the water to the original level, and by July 16 many of the oldest of the newly formed sporocarps were blasting. On July 21 I again lowered the water to 1^{cm} in depth, and by July 25 the oldest of the sporocarps, formed after that date, had safely passed the period where blasting had occurred. I then put on 20^{cm} of water and found that the blasting occurred after the sporocarp had been visible only about two days, instead of four to six days as observed at the 10^{cm} level. I imbedded and sectioned many of these sporocarps in order to determine the relative vitality of megaspores and microspores. It was from a study of these that I conceived the idea of blasting the megaspores and continuing the development of the sporocarps containing only the surviving microspores. I shall speak later of the methods employed.

I was at a loss to know just what had caused the blasting. While mere depth of water seemed to be the immediate cause, several factors were involved, such as oxygen supply, intensity of light, and temperature, all of which were varied with the change in depth. On August 5 I moved this tank, which had been in subdued light, to the east side of the greenhouse into strong light, and threw a mist of tap water over the plants in order to keep them saturated, but at the same time aerated. This water was siphoned off constantly, so that most of the sporocarps were at the surface. In a few days (August 11) I noted that blasting was occurring quite as universally as before. While the plants now had more light and a better oxygen supply than before, I had lowered the temperature during the middle of the day about 8° and at night about 3°. It was plain that this variation in the

temperature was a very important factor. I then grew the plants out of water for a week without applying the spray, and had no trouble with blasting. This process was repeated several times with the same

TABLE II

OPEN AIR TEMPERATURE READINGS TAKEN FROM 2:00 P. M. TO 3:00 P. M.

1907	Weather	Open air	Pond surface	15 ^{cm} under water	Tank	Dry soil	Wet soil
August 21.....	cloudy		20	23.5			
August 22.....	clear		23		25		
August 23.....	clear	21	20	20	21.5		
August 24.....	clear	..	25	21.5	27		
August 25.....	clear	24.5	27	20	28	35.5	31.5
August 26.....	cloudy	24	22.75	19	24	23	24
August 27.....	cloudy	22.5	23	20.5	24	24	23.5
August 28.....	clear	24	26	22	27.25	29	28
August 29.....	cloudy	20	21	19.25	21	..	22
August 31.....	cloudy	24	28	23	28.5	30.5	
September 1.....	clear	33.5	30.75	25.5	33.5	33	
September 14....	clear		26	21	27		
Average readings		24.18	24.37	21.39	26.06	29.16	25.8

TABLE III

GREENHOUSE TEMPERATURE READINGS TAKEN FROM 2:00 P. M. TO 3:00 P. M.*

1907	Weather	Air	SOUTH TANK		NORTH TANK			
			Wet soil	Surface	Surface	Tap	Jet	Spray
August 19.....	clear	34		35.5	30	15.5		
August 20.....	cloudy	28.5			20.5	16		
August 21.....	cloudy	25						
August 22.....	clear	30				19	30	25
August 23.....		26		24	23			
August 24.....	clear	30	32	32	23.5	24	34	24.5
August 25.....	clear	27.75	29.75	29.5		21	25.5	21.5
August 26.....	cloudy	29	27	27.25	23.5	19.5	31	25
August 27.....	cloudy	25		24.5	22	18.75	24	23
August 28.....	clear	30	30.5	28	26	19.5	31	23.5
August 29.....	cloudy	22	22	21.5		20	22	20.5
August 31.....	cloudy	33	33	30	28.5	19	35.5	28
September 1.....	clear	39.5	35.25	34.5	33		41	26
September 14.....	clear	32.75		30	27		34	28
Average readings		29.45	29.93	28.8	25.7	19.22	30.8	24.5

*It will be noted that this table of readings was taken in 1907. It is more complete than the one taken in 1905, but does not vary materially from it, except in the columns marked jet and spray. These are higher in this table because the water was heated by means of the sun which shone on about 12^m of the pipe, raising the average reading for the jet 9°, and for the spray 4°. The spray was in the north end of greenhouse in 1905, close to the riser pipe; and the temperature of the jet was very little above the tap in this place. (See diagram of piping.)

results. By this time I had discovered that the megaspores were the first to succumb when the plants were chilled, and I also found an occasional sporocarp, among those which had not apparently been blasted, which showed that the megasporangia and oldest set of microsporangia had been killed, but the younger microsporangia were in a healthy state (*fig. 13, b, c*). So far as I could determine, these sporocarps were in a fair way to reach maturity, notwithstanding the fact that the megaspores were all dead. I then shortened the time of application of the spray, in order to determine the shortest period which would kill the megaspores and yet not blast the entire sporocarp. This I found to be no easy task, as it is not possible to determine exactly, from external appearance, the stage of development of the sporogenous tissue. I was able finally to be reasonably sure from

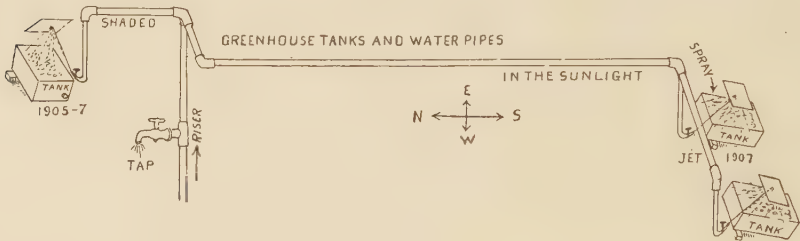


Diagram of piping and position of tanks in greenhouse

their size and thickness when sporocarps had reached the mother cell stage. In such sporocarps, under the conditions of this experiment, the application of the cold spray day and night for 48-60 hours would not cause the entire sporocarp to damp off, but most of the sporocarps when sectioned showed only traces of megaspores in the sporangium. It was in these sporocarps that I got such a great variety of forms of microspores (*figs. 15-22*).

The sporocarps treated less than 48 hours generally developed normally, while those treated for a longer period than 60 hours generally damped off. I was able to treat plants in August and September 1907, in the south end of the greenhouse, in stronger light and at a higher temperature (see table) for a much longer time (76-96 hours) before blasting occurred. Also in the same year I was able to grow abundant and normal sporocarps in tanks out of doors, at temperatures shown in the table, in as much as 20^{°C} of water. At the same

time, plants in the same tank, in 8 to 12^{cm} of water, at the same temperature but shielded from the direct rays of the sun, either blasted completely or showed more or less abortion among the megaspores and occasionally enlargement of some of the microspores. It is evident, therefore, that the plants are dependent on strong light and high temperature in order to mature perfect sporocarps, and that water at any depth less than 20^{cm} need not cause blasting unless it greatly lowers the temperature or reduces the light below a certain minimum.

Discussion

A close inspection of this process of blasting brought to light the important fact that under the conditions of growth to which these plants were subjected the megaspore was the first to show signs of abortion. It might be added that through a long list of experiments on the younger stages of sporocarps this was the invariable rule. Unfavorable conditions of growth, whether brought about by depth of water, lack of light, low temperature, or drought, without exception always affected the megaspores more than the microspores. Later experiments revealed the fact that in the more mature sporocarps this was not the case. Hard conditions brought on after the megaspores are well formed, but about the time the microspores are in the mother cell stage, will produce abortion among the microspores. The critical period in the life of the spore, whether microspore or megaspore, seems to be from the time when the mother cell comes into the synapsis stage until the tetrads are well on the way to becoming spores. This also agrees with Miss PFEIFFER's observations on *Azolla* (20). However, it proved to be much easier to arrest the development of the megaspores than to affect the microspores in this way, and when abortion did occur it was much more nearly universal in the case of the megaspores than in that of the microspores.

Excellent examples were secured showing sporocarps without a single megaspore, while I was never able to secure the complete blasting or abortion of the microspores alone. Many experiments were made in which both were completely blasted, but if either survived it was always the microspores, though sometimes only a small number. It was also noticeable that when the sporocarps were subjected to such treatment as to blast the megaspores in their early

stages, sometimes, when the plants were afterward placed under the best of growing conditions, only part of the microspores survived the change. The surviving ones would then respond with vigor, becoming sometimes three times the usual size for the normal microspores (figs. 26, 28). It was noticed also that when a sporocarp, whose oldest sporangia were just forming tetrads, was subjected to a spray of cold water for several days, the megaspores would abort, and the microspores, which at this time would be just coming into the mother cell stage, would not undergo the reduction division while under the spray, but would continue to grow until they became much larger than the ordinary microspores, assuming at the same time many unsymmetrical and unusual shapes (figs. 15-22). When these were placed later in good growing conditions, both reduction divisions occurred without the formation of more than mere traces of walls (fig. 14, c), but the entire wall of the mother cell became much heavier. Repeated attempts at germination under the most favorable conditions have so far failed to demonstrate what these spores might become ultimately. The failure to germinate seems to find its explanation, not in any want of vitality of the cell, but rather in the rigidity and continuity of the surrounding wall. As is well known, the heavy walls of both megaspores and microspores are interrupted at the papilla, which has only a light membranous covering, due to the presence of radiating protoplasmic strands (figured in SACHS 22 for *Pilularia globulifera*) that hold the four spores in close proximity until the wall is well developed over the free surfaces. The tapetal layer always incloses the tetrad, but forms a perinium only where it comes in contact with the cell membrane of the functioning spore. It is prevented from touching this wall at the papilla by the other three members of the tetrad (figs. 6-10), yet the perinium will not form around an aborting spore. The microspores separate earlier than the megaspores, hence the papilla is less evident in them than in the megaspores where the abortive members of the tetrad may not be cast off at all, often remaining until germination has begun. When the tetrad divisions are delayed, there is not a corresponding delay in wall formation, which goes forward at such a rate that when the divisions occur the wall is too strong to disrupt, though in the second division evanescent cell plates are sometimes evident (fig. 14, c).

As there is nothing to interfere, the wall is laid down continuously over the entire surface of the cell, being thicker than the ordinary microspore wall, with no thin places, and absolutely unresponsive to germinative conditions. Cases of delayed or estopped germination due to resistant seed and spore coats are not uncommon, as shown by CROCKER (15) in the cocklebur and other plants.

As said before, unfavorable conditions in the early life of the sporocarp produced blasting of the megaspores, and sometimes delayed the reduction divisions in the 16 mother cells. However, unfavorable conditions at a later date permitted a very complex set of results, most of the megaspores blasting, while some of the microspore mother cells would undergo division and separate into spores (*fig. 21*). An interesting condition was obtained by blasting just as the microspore tetrads were formed, and then transferring the plants to the best condition for growth. In this case a few microspores would survive in a few of the sporangia, growing with astonishing vigor, some of them reaching a size quite beyond the ordinary microspores (*figs. 24-27*). We have in this case an intermediate sized spore, sufficiently unlike the microspore to warrant the question whether the abortion of tetrads among the megaspores did not begin in this way. The difference in size between the ordinary microspores and these unusual ones is as great as that shown between the reputed microspores and megaspores of *Calamostachys* and *Sphenophyllum*.

Probably the most interesting of all the results were obtained by growing *Marsilia* in subdued light during the usual time of sporocarp-formation (July and August), and then forcing the plants to develop sporocarps at a later period. I had previously determined that although the plants grew luxuriantly in subdued light, they would not produce sporocarps. On August 15, 1906, plants were moved to the south end of the greenhouse and given the maximum amount of heat and light. My intention in this experiment was to throw all the vigor of the plant into a few sporocarps and then to inhibit further sporocarp-formation by means of a spray of cold water. A few sporocarps appeared by September 10, which was more than two months later than the usual time for sporocarp-formation. These grew with great vigor until the occurrence of exceptionally cool and cloudy weather, which began September 19. After this time no more sporo-

carps appeared, and all the younger ones, already in evidence, began to blast, although I did not use the spray. Sections of these showed, as usual, that the megasporangia were the first to succumb to the unfavorable conditions. Sections of the older ones showed a condition which I had not seen before, namely that the blasting was much more general in the microsporangia, and that in many of the oldest sets of microsporangia megaspores were developing (*figs.* 32-37); while in a few microsporangia abortion had proceeded until only one microspore, which was 8-16 times larger than the ordinary ones, remained (*figs.* 28, 31). The few remaining sporocarps were carefully grown to maturity, and on dissection showed a number of sori containing two megaspores instead of one, as I had found in all the cases thus far noted. That these secondary forms (called secondary because of forming at a later date than the original or primary megaspores, and also because of being smaller; *figs.* 32-43) were megaspores there could be no doubt. Every distinguishing character peculiar to the megaspore was present, such as size, shape, thickness of wall, position and shape of nucleus (which is always apical and meniscoid), and manner of vacuolation. There could be no doubt that these forms, though occurring in microsporangia, were far beyond any microspore in special characters; but they still retained some undoubted microspore characters; such as an approach to the general spherical form, the long axes of the smaller (intermediate) size being proportionally shorter than those of the primary megaspores (*figs.* 38-43). So far I have failed to induce germination in any of these induced forms.

A number of the enlarged microspores from this culture were sectioned and showed certain characters belonging to megaspores. The ordinary microspore nucleus is spherical and central, while that of the megaspore is generally meniscoid and apical. It was easy to secure transition forms of nuclei among these microspores, which ranged from spherical, central ones in the case of the smaller spores, through a series of spheroidal, parietally placed nuclei in larger spores (*fig.* 30), until in the largest sizes, which were also more vacuolated, there was always found a meniscoid nucleus in the apical region of the slightly elongated spore (*fig.* 31).

The microsporangium represented in *figs.* 28, 29 shows several

functionless tetrads, which, while now distorted and shriveled, must have drawn heavily upon the supply of nutrition. This may explain why the largest microspores, while acquiring certain megaspore characters, were not able to attain more fully to megaspore proportions. In examining the sporangia that contained secondary megaspores, I was never able to find these large shriveled tetrads. This shows that all the tetrads must have aborted during the early stages of development, thus permitting the whole of the nutrition to be concentrated in this one spore, as is the usual case in primary megasporangia. *Fig. 36* is unusual for secondary megaspores, and shows in addition to the successful spore a single member in each of the three tetrads slightly enlarging. *Fig. 37* is more usual, and shows five tetrads which aborted much earlier. Two cultures were grown in 1907 under conditions similar to those just described, and the same results were obtained. Occasionally in all of these cultures I found megasporangia containing well-developed microspores (*figs. 2, 33, 35, 37*). In ordinary plants these were always found on the periphery of the sporogenous area and appeared to be related to the food supply. Scant nutrition may cause the sporogenous tissue in some megasporangia to revert to the probable ancient homosporous condition.

This production of secondary megaspores I believe to be as closely related to nutrition as is the formation of primary megaspores. In numerous cultures I was able to observe that the formation of megaspores is possible only when the plants are well nourished. In the cultures in which I secured secondary megaspores, I always had long and strong rhizomes and allowed only a few sporocarps to form just at the end of or after the fruiting season. Then by cutting off all later sporocarps I was able to throw all the energies of the plant into a very few. By this means I was able to secure not only the most marked abortion and enlargement of the microspores, but also the formation of secondary megaspores, which, since they occur in microsporangia, would undoubtedly have developed, under ordinary conditions, as microspores.

In a plant so plastic as *Marsilia*, it is not difficult for one to conceive how the heterosporous habit may have become fixed. If excessive nutrition will now cause certain microsporangia to develop megaspores, and excessively hard conditions will cause the plant to

revert to the homosporous habit, it would seem reasonable to conclude that the plant had attained to heterospory gradually, a few of the better nourished sporangia each developing a few spores slightly larger than the rest. Other things being equal, these spores in germinating would naturally develop the larger prothallia, because of a larger amount of food material within the spore. We might expect that these prothallia would bear archegonia. Thus the determination of the sex of the prothallia would be at once shifted from dependence on the chance external conditions after the spore germinates, as found in the homosporous ferns, to dependence on nutritive conditions while the spore is being formed. This, it appears to me, must have been the process by which heterospory made its appearance in Marsilia.

I should also state that megaspores were noticed which were packed unusually full of starch grains (*figs. 44, 45*). These forms were larger than any megaspores that I have seen and had developed no perinium. I was not able to observe stages in their development nor to account for the phenomenon.

Theoretical discussion

If the doctrine of recapitulation be applied here, we may say that Marsilia can be so manipulated as to show in the development of its sporocarps the various stages in the production of complete heterospory, through the abortion of many spores and the special nutrition of a few.

We know that the heterospory described by WILLIAMSON and SCOTT in such forms as *Calamostachys Casheana* began in a very modest way, the diameter of the megaspores being not more than three times that of the microspores in the most extreme cases. We are also told that in the genus *Calamostachys*, *C. Binneyana* is homosporous and *C. Casheana* is heterosporous; but even in *C. Binneyana* abortive spores are found, and the remaining spores are larger in proportion as fewer develop in each tetrad, the diameter being fully twice as great when only one develops as when three remain.

WILLIAMSON and SCOTT point out that in *C. Casheana*, also, abortion is evident, but is confined to the megasporangia; in fact, their figures show no abortion in the microsporangia. They conclude, therefore, that heterospory was reached by the abortion of the

spores in certain sporangia; then better nutrition caused the excessive development of the survivors at the expense of the aborting ones; and this process continued until specialized spores (megaspores) were formed.

Heterospory, as found in *Calamostachys*, is in its incipiency. We see its very beginnings in *C. Binneyana*, where all the sporangia show a tendency toward it by the promiscuous abortion of some members of all the tetrads, with the corresponding enlargement of the survivors. In *C. Casheana* we see it feebly established, abortion being confined to certain sporangia, yet fully three-fourths of them showing abortive spores quite variable in size.

In *Marsilia*, in ordinary plants, heterospory is much more firmly established, abortion being confined to not more than one-fifth or one-sixth of the sporangia, and at maturity the megaspores are very nearly equal in size, and many times larger than the microspores. But when by any means the fertility of these megaspores is destroyed, there is still a very large number of potential spores in the original homosporous condition and sufficiently young to respond to the stimulus given them by the added nutrition which should have gone toward maturing the megaspores. We then see the plant repeating its probable ancient habit of developing some of the spores to greater size at the expense of the others; and we have repeated in every detail the processes observed in *C. Binneyana*, which is homosporous. Again, well-nourished plants, allowed to produce only a limited number of sporocarps, carry this abortion and enlargement still farther, and a few sporangia produce still larger spores, varying as much in size as in *C. Casheana*, which is heterosporous. *Figs. 32-43* show that in many sporangia abortion and enlargement continue much farther than in *C. Casheana*, in that an intermediate sized megaspore is produced. This adds more evidence as to the plasticity of the plant and the manner in which heterospory may have originated in it.

Summary

1. It is possible by means of a spray of cold water to kill the megaspores of *Marsilia*, which occur only in the oldest sporangia, and then by putting the plant under good conditions to mature sporocarps without megaspores.

2. The greatest variations occur when the megaspores and the oldest microspores are blasted, and when strong plants develop a few sporocarps out of season.

3. Enlargement does not appear among the microspores when less than half the spores abort, and the surviving spores are larger the greater the amount of abortion.

4. The mother cells may be checked in their development till the tapetal nuclei completely invest them. A perinium will then form around the mother cell wall, which invests the four young spores. In such cases the sporangium invariably contains sixteen large forms each containing four nuclei. At other times, when growth is less checked, the spores are more or less completely free and show great variation in size and shape.

5. The contest for supremacy among the young megaspores of each sporangium is very evident, many of them assuming considerable proportions, but one, centrally located, invariably secures the ascendancy. Sometimes the contest is very close between two or more members of the same tetrad. Very often the surviving member will carry attached to its papilla the aborted members even to germination.

6. The enlarged microspores vary in size, the largest being 8-16 times the size of the ordinary ones; the position of the nucleus changing from a central (usual) to an apical one, as in the megaspores. As vacuolation is more extensive, the shape of the nucleus also varies from the usual spherical form to the oval, and finally in the largest to the meniscus shape, as in the megaspore.

7. In extreme cases of abortion in the microsporangia only one spore survives, which is about 16 times as large as the normal microspore. The aborted tetrads remain as in the megasporangium, but are much larger and better developed, thus showing a sharper and more prolonged contest for supremacy.

8. In plants kept from sporocarp-formation until September 10, many microsporangia developed secondary megaspores, so called because they are formed after the first or primary ones. Such megaspores are intermediate in size and are also more nearly the spherical shape of the microspores.

9. A few cases were noted in which the megaspores did not

develop a perinium, but enlarged considerably and became gorged with starch.

10. In the normal plants, and in all cultures, a close examination reveals a homosporous tendency, shown by the formation of microspores in the megasporangia, especially in those most distant from the nutritive supply.

11. Marsilia may be made to repeat, under culture, all the phases in the development of heterospory reported by WILLIAMSON and SCOTT for *Calamostachys Binneyana* and *C. Casheana*, and in addition to produce a megaspore of intermediate size.

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UNIVERSITY OF IDAHO
MOSCOW

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EXPLANATION OF PLATES III-VI

All drawings were made with a Spencer camera lucida. In all drawings showing nuclear detail a Zeiss 2^{mm} apochromatic objective with compensating oculars was used. The drawings and photomicrographs were then reduced to the magnifications indicated in the explanation of figures.

PLATE III

FIG. 1.—Diagrammatic sectional view of sporocarp; *A*, *B*, *C*, *D* designate planes; *C'*, soral pad attached to vascular bundle and subtending a single sorus. $\times 7$.

FIG. 2.—Portion of a section showing three sporangia of the oldest series (right) containing megasporae, and one (left) containing microspores. $\times 40$.

FIG. 3.—Young sporangium, showing 8 of the 16 mother cells in synapsis, just as the tapetum begins to form the plasmodium. $\times 292$.

FIG. 4.—Older sporangium, showing tetrads with the tapetal plasmodium dispersed among them. $\times 292$.

FIG. 5.—First stage in which one can determine that the sporangium is to contain megaspores; several spores are enlarging, but the mass of tapetal nuclei is collected about the larger one in the center. $\times 292$.

FIGS. 6–10.—Showing the competition among the members of the tetrad and the persistence of the three aborting members through the various stages of megaspore formation; they are often evident even after germination, as shown in *fig. 10a*. *Figs. 6, 7, 8, 9*, $\times 300$; *10b*, $\times 85$.

PLATE IV

FIG. 11.—Photomicrograph showing the aborted members of the tetrad still attached to the papilla of mature megaspore. $\times 85$.

FIG. 12.—Diagrammatic representation of fruiting rhizomes, showing the effect of varying the depth of water when the plants are in such subdued light as to be just able to produce sporocarps; *b*, blasting; *m*, maturing.

FIG. 13.—Condition of various sporangia of a sorus during the process of blasting; *a*, old aborted microsporangium, megasporangium in the center; *b*, *c*, young microsporangia from same sorus.

FIG. 14.—*a*, normal microsporangium; *b*, sporangium in which the 16 mother cells have not formed walls during the tetrad divisions; *c*, same in section; *d*, sporangium with extensive abortion, a few of the surviving spores enlarged.

FIG. 15.—Portion of a section of a sporocarp in which all the megaspores have aborted, as shown by the empty megasporangia, *m*; in the microsporangia no walls have been formed in the tetrad divisions, so that each sporangium contains but 16 enlarged and irregular bodies instead of the usual number of 64 spherical spores. $\times 55$.

FIGS. 16, 17.—Single entire sporangia, showing the 16 bodies as in *figs. 14b*, and *15*. $\times 105$.

FIG. 18.—Three sections, *a*, *b*, *c*, of various bodies from sporangia as in *figs. 16, 17*, showing nuclear structure and behavior. $\times 400$.

PLATE V

FIGS. 19, 20.—Similar to *fig. 18*; forms more irregular. $\times 400$.

FIG. 21.—Microspores resulting from tetrads which have had varying success in forming walls during the second division, due to unfavorable conditions of growth. $\times 133$.

FIG. 22.—Nuclear structure and behavior in earlier stages of such microspores. $\times 400$.

FIG. 23.—A normal microsporangium at maturity, containing 64 spores. $\times 95$.

FIGS. 24, 25.—Microsporangia in which many of the microspores have

aborted, showing at maturity a number of normal spores and a single enlarged microspore. $\times 95$.

FIG. 26.—More complete abortion of microspores, with four enlarged. $\times 95$.

FIG. 27.—Same more highly magnified.

PLATE VI

FIG. 28.—Microsporangium showing a few remaining but abortive tetrads, and only one (much enlarged) microspore. $\times 95$.

FIG. 29.—Same more highly magnified.

FIGS. 30, 31.—Sectional views showing internal and external transitions from the ordinary spherical microspore, which has a central nucleus and no vacuolation, toward the megaspore form and condition; *fig. 30* shows an enlarged, spherical, but vacuolated spore, with spheroidal nucleus laterally placed; *fig. 31*, a more enlarged, spheroidal spore, more vacuolated, with meniscoid nucleus apically placed. $\times 180$.

FIG. 32.—Section of sporocarp showing primary (*p*) and secondary (*s*) megaspores, as determined by age, position, size, and stage of wall formation. $\times 33$.

FIG. 33.—Same showing three primary (*p*) and two secondary (*s*) megaspores, and one megasporangium, *m*, containing microspores. $\times 33$.

FIG. 34.—Same, showing one central and one lateral section of primary megaspores, *p*, and six secondary megaspores, *s*. $\times 33$.

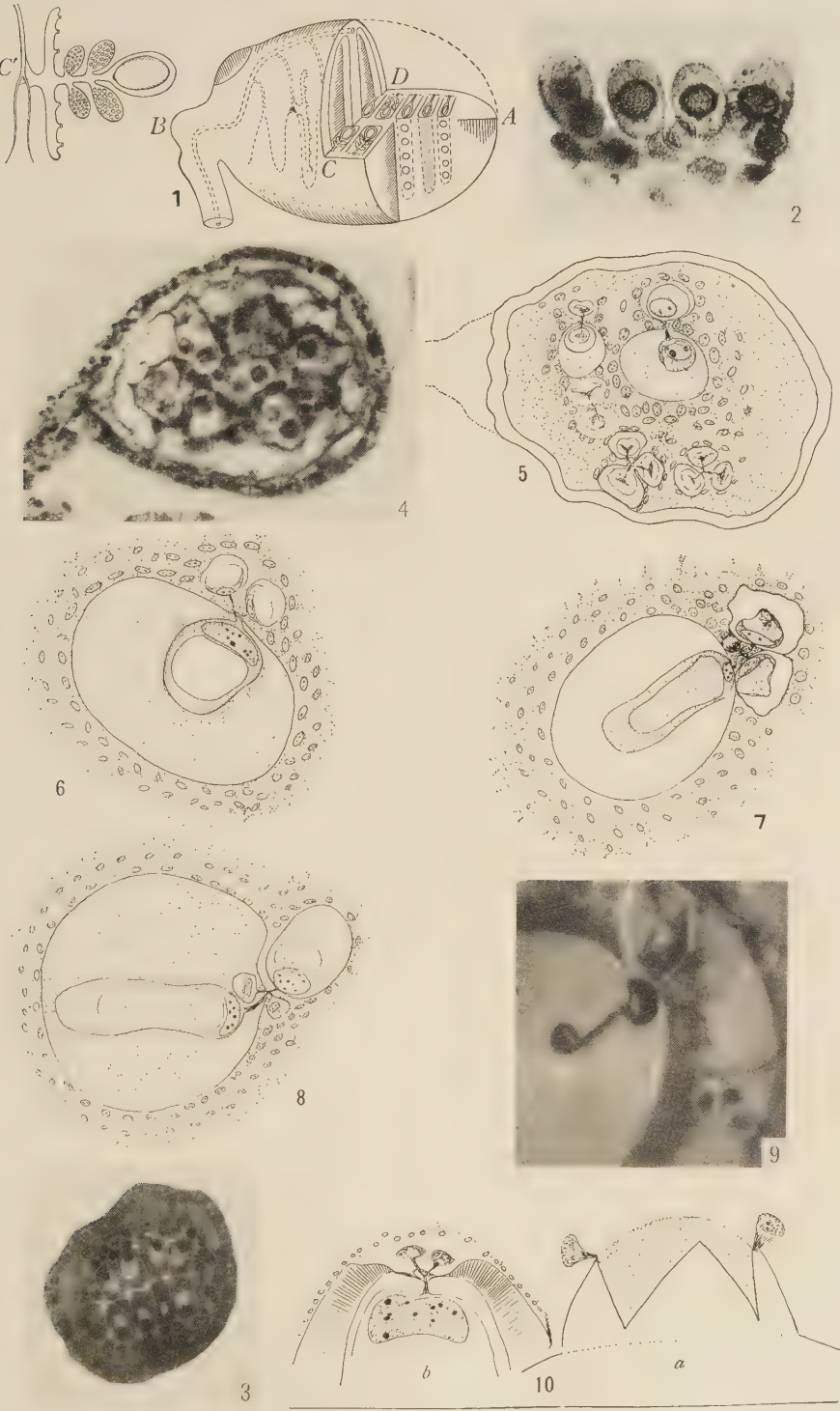
FIG. 35.—Same showing two primary (*p*) and two secondary (*s*) megaspores, and one primary megasporangium, *m*, containing normal and aborting microspores. $\times 33$.

FIG. 36.—Primary and secondary megaspores more highly magnified; the secondary megasporangium showing three sets of tetrads in which one of the spores in each set has enlarged and become vacuolated (not marked in the figure), thus competing sharply with the functioning megaspore, *s*, for supremacy. $\times 87$.

FIG. 37.—Primary megasporangium, *n*, containing microspores nearly mature, one of the oldest microsporangia, *o*, containing a young secondary megaspore, the same age as that shown in *fig. 36*; the microspores are the same age as the primary megaspore in *fig. 36*. $\times 87$.

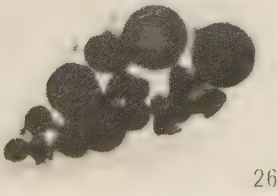
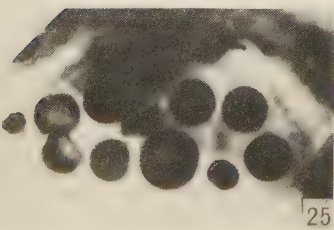
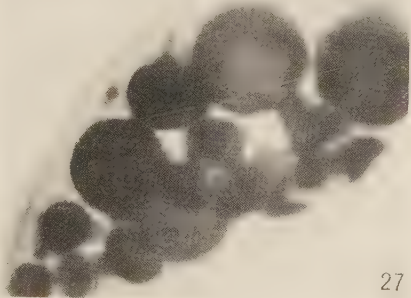
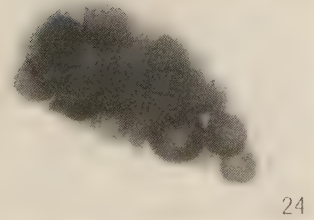
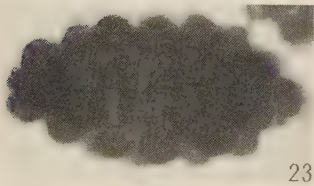
FIGS. 38-43.—Variation in size and shape between the primary (*p*) and the secondary (*s*) megaspores at maturity. $\times 44$.

FIGS. 44, 45.—Enlarged megaspores packed full of starch grains, but having no perinium. $\times 44$.

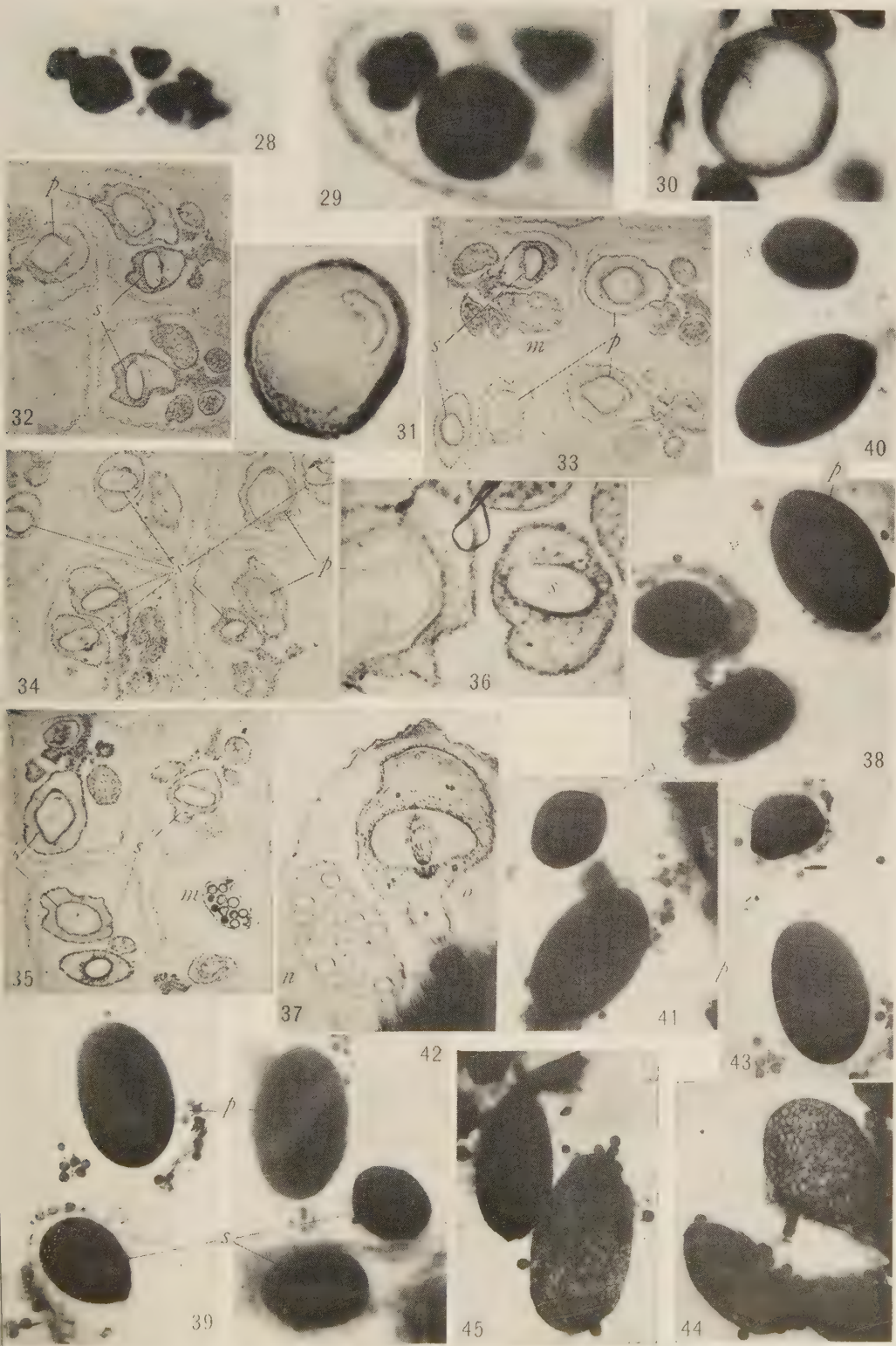




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